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REVIEW ARTICLE

Migrating Schwann cells direct axon regeneration within the peripheral nerve bridge

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Abstract

Schwann cells within the peripheral nervous system possess a remarkable regenerative potential. Current research shows that peripheral nerve-associated Schwann cells possess the capacity to promote repair of multiple tissues including peripheral nerve gap bridging, skin wound healing, digit tip repair as well as tooth regeneration. One of the key features of the specialized repair Schwann cells is that they become highly motile. They not only migrate into the area of damaged tissue and become a key component of regenerating tissue but also secrete signaling molecules to attract macrophages, support neuronal survival, promote axonal regrowth, activate local mesenchymal stem cells, and interact with other cell types. Currently, the importance of migratory Schwann cells in tissue regeneration is most evident in the case of a peripheral nerve transection injury. Following nerve transection, Schwann cells from both proximal and distal nerve stumps migrate into the nerve bridge and form Schwann cell cords to guide axon regeneration. The formation of Schwann cell cords in the nerve bridge is key to successful peripheral nerve repair following transection injury. In this review, we first examine nerve bridge formation and the behavior of Schwann cell migration in the nerve bridge, and then discuss how migrating Schwann cells direct regenerating axons into the distal nerve. We also review the current understanding of signals that could activate Schwann cell migration and signals that Schwann cells utilize to direct axon regeneration. Understanding the molecular mechanism of Schwann cell migration could potentially offer new therapeutic strategies for peripheral nerve repair.

KEYWORDS

axon regeneration, migration, nerve bridge, Schwann cell, signals, transection injury

1 | INTRODUCTION

In contrast with the central nervous system (CNS), the peripheral nervous system (PNS) has a remarkable ability to regenerate following injury, and Schwann cell plasticity contributes significantly to this capacity (Boerboom, Dion, Chariot, & Franzen, 2017; Carr & Johnston, 2017; Jessen & Mirsky, 2005, 2016, 2019a). Schwann cells in adult nerves are a regenerative cell type and they retain the ability to revert to a repair competent state following nerve injury (Jessen & Mirsky, 2019). Studies have shown that, among others, the transcription factor cJun, as well as Merlin-dependent Hippo signaling play major roles in reprogramming adult Schwann cells into a repair

competent cell (Arthur-Farraj et al., 2012; Fontana et al., 2012; Mindos et al., 2017; Schulz et al., 2016).

In response to injury, the area of nerve distal to the site of injury undergoes a series of changes known as Wallerian degeneration, and creates a permissive environment for subsequent axon regeneration (Coleman & Freeman, 2010). Schwann cells in the distal nerve stump break down their myelin and activate a program called myelinophagy for myelin clearance (Brosius Lutz et al., 2017; Gomez-Sanchez et al., 2015; Li et al., 2020). Schwann cells also play an active role in axon degradation within the distal nerve, remodeling their actin cytoskeleton to produce actin spheres that accelerate axon clearance (Vaquie et al., 2019). Within 6 hr of injury, Schwann cells in the distal nerve stump also produce and secrete pro-inflammatory factors to recruit macrophages to further accelerate clearance of axonal and myelin debris (Martini, Fischer, Lopez-Vales, & David, 2008; Stratton et al., 2018; Zigmond & Echevarria, 2019). Following their dedifferentiation, Schwann cells proliferate and extend longitudinally to form bands of Büngner to help guide axon regrowth; they also secrete cytokines, growth factors, neurotrophins and extracellular matrix molecules to promote neuronal survival and axon regeneration (Jessen & Mirsky, 2016; Ma, Hung, & Svaren, 2016). Stimulated by such retrograde injury signals, cytokines, chemokines, growth factors, and neurotrophins, injured neurons rapidly activate an intrinsic program and regenerate quickly toward their distal targets (Abe & Cavalli, 2008; Navarro, Vivo, & Valero-Cabre, 2007; Rishal & Fainzilber, 2010). Upon the contact with regenerating axons, Schwann cells remyelinate regenerated axons and repel infiltrated macrophages to complete the process of peripheral nerve repair (Fry, Ho, & David, 2007; Jessen & Mirsky, 2016).

Current evidence shows that peripheral nerve-associated Schwann cells promote the repair of multiple tissue types including peripheral nerve gap bridging, skin wound healing, and regeneration of both digit tips and teeth (Carr & Johnston, 2017; Johnston et al., 2016; Jones et al., 2019; Parfejevs et al., 2018). The injury-induced changes not only reprogram denervated Schwann cells into a repair-competent state, but may also give them a highly motile phenotype. These Schwann cells then not only migrate into the area of injury to become a key component of regenerating tissue, but also secrete growth factors to activate local mesenchymal stem cells to drive tissue regeneration (Johnston et al., 2016; Jones et al., 2019). The importance of migratory Schwann cells in tissue regeneration is perhaps most evident in the case of a peripheral nerve transection injury (Cattin et al., 2015; Dun et al., 2019; Parrinello et al., 2010). Following nerve transection, Schwann cells at the tips of the severed nerve stumps (i.e., distal tip of the proximal stump and proximal end of the distal stump) undergo a process of dedifferentiation, followed by proliferation and migration into the nerve gap where they form Schwann cell cords and bridge the transected nerve stumps. The formation of Schwann cell cords is key to successful peripheral nerve regeneration (Cattin et al., 2015; Chen, Chen, Parkinson, & Dun, 2019; Dun & Parkinson, 2020; Parrinello et al., 2010).

This review article will focus on the behavior of Schwann cell migration from the transected nerve ends into the nerve bridge and discuss how migrating Schwann cells direct regenerating axons toward and into the distal nerve stump. We will also review the current understanding of signals that are proposed to activate Schwann cell migration and mechanisms that Schwann cells utilize to direct axon regeneration. We have recently reviewed signaling by axon guidance molecules and its potential role in regulating the trajectories of Schwann cell migration in the nerve bridge (Dun & Parkinson, 2020); therefore, these aspects will not be covered in this present review article.

2 | FORMATION OF NEW NERVE TISSUE FOLLOWING INJURY, THE NERVE BRIDGE

2.1 | The nature of variable lengths of nerve gap following transection

The rodent sciatic nerve transection has been widely used a research model to understand the molecular mechanisms of peripheral nerve regeneration after injury (Savastano et al., 2014). However, researchers in this field have experienced that, with no attempted re-suturing of the nerve ends, the lengths of the nerve gaps are variable following transection (Bozkurt et al., 2011; Cattin et al., 2015; Chen et al., 2019; Dun & Parkinson, 2018a; Parrinello et al., 2010; Savastano et al., 2014). Like the majority of the peripheral nerves in mammals, the rodent sciatic nerve is under tension and therefore a nerve gap will be generated upon transection injury due to the retraction of the nerve ends (Chen et al., 2019; Dun & Parkinson, 2018a). In our experience, we normally create a nerve gap varying between 1 and 2.5 mm (Figure 1a–c) (Chen et al., 2019; Dun et al., 2019; Dun & Parkinson, 2015, 2018a, 2018b) and these gaps may first be bridged by a fibrin deposit (Schroder, May, & Weis, 1993; Williams, Longo, Powell, Lundborg, & Varon, 1983). Perineurial cells are the first cell type from both severed nerve ends migrating into the nerve gap (Martini, Schachner, & Faissner, 1990; Schroder et al., 1993; Weis, May, & Schroder, 1994). Subsequently, nerve fibroblasts, endothelial cells, and Schwann cells migrate into the nerve gap and form new tissue to connect both nerve stumps. This newly formed tissue is now known as the "nerve bridge" (Cattin et al., 2015; Dun et al., 2019; Dun & Parkinson, 2015, 2020; Parrinello et al., 2010). The nerve bridge serves as a substrate for regenerating axons crossing the nerve gap.

In some cases of rodent sciatic nerve transection using a scissors, the sciatic nerve could be detached from the attaching muscle during the surgical procedure of transection, which often results in a further retraction of both nerve ends and generates a longer gap. Under such circumstances, a fibrin deposit cannot form to connect both nerve ends due to the leakage of the fibrin clot in the larger nerve gap (Belkas, Shoichet, & Midha, 2004; Lundborg, Dahlin, et al., 1982; Zhao, Dahlin, Kanje, & Lundborg, 1993); therefore, a nerve bridge does not form and regeneration cannot occur in such a large nerve gap without surgical repair. In our work using the proteolipid protein-green fluorescent protein (PLP-GFP) mice which labels Schwann cells with GFP (Mallon, Shick, Kidd, & Macklin, 2002), we observed that a

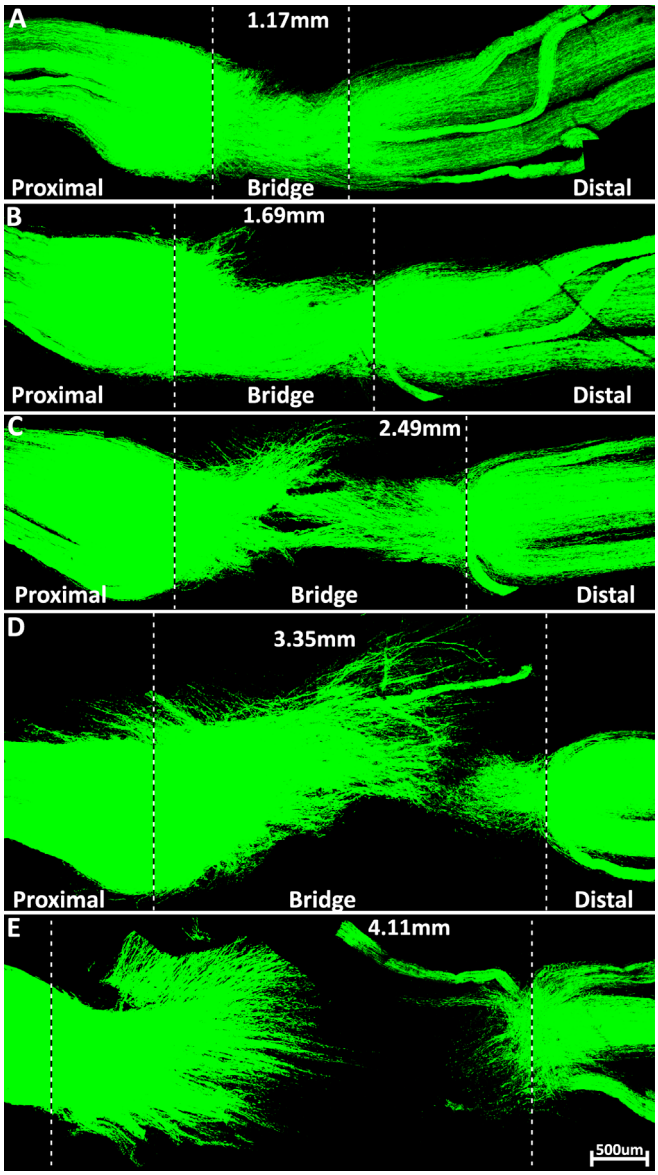


FIGURE 1 The length of the sciatic nerve gap affects Schwann cell cord formation within the nerve bridge. Z-stack images were captured using confocal microscopy with reconstruction from whole sciatic nerve preparations 14 days after transection injury in PLP-GFP mice (GFP+ Schwann cells). Dashed lines indicate the cut ends of both proximal (left) and distal (right) nerve stumps. (a) Schwann cell cords connecting the proximal and the distal nerve stumps in a 1.17 mm sciatic nerve gap. (b) Schwann cell cords connecting the proximal and the distal nerve stumps in a 1.69 mm sciatic nerve gap, some Schwann cells have left the nerve bridge in the proximal nerve stump. (c) Schwann cell cords connecting the proximal and the distal nerve stumps in a 2.49 mm sciatic nerve gap with more Schwann cells observed leaving the nerve bridge in the proximal nerve stump. (d) With a larger sciatic nerve gap (3.35 mm), only a few Schwann cell cords connect the proximal and the distal nerve stumps. (e) In a 4.11 mm sciatic nerve gap, migrating Schwann cells are unable to form Schwann cell cords and do not connect both nerve stumps to form a nerve bridge. GFP, green fluorescent protein; PLP, proteolipid protein

gap distance of greater than 3.5 mm between the nerve stumps was a barrier to correct nerve bridge formation (Figure 1d,e).

2.2 | Stages of nerve bridge formation

We, and others, typically use mouse and rat sciatic nerve transection as a research model to create nerve gaps and study the molecular and cellular events regulating axon regeneration across such peripheral nerve gaps (Chen et al., 2019; Dun et al., 2019; Dun & Parkinson, 2015, 2018a; Parrinello et al., 2010; Savastano et al., 2014). Following a sciatic nerve transection, numerous erythrocytes, granulocytes, thrombocytes, and macrophages are localized into the nerve gap (Schroder et al., 1993; Weis et al., 1994; Williams et al., 1983). These cells are the earliest cell types arriving into the nerve bridge, and they are released from the transected endoneurial and epineurial blood vessels of the nerve rather than migration into the nerve gap from either nerve stump. In rat sciatic nerve transection injury, the nerve gap is first bridged by a fibrin deposit between the distal and the proximal nerve stumps at 2 days following transection (Cattin et al., 2015). At Day 2 postinjury, the nerve bridge is composed of approximately 50% macrophages, 24% neutrophils, 13% fibroblasts, 5% endothelial cells, and 8% other cell types (Cattin et al., 2015). Perineurial cells respond rapidly to nerve transection and they are the earliest cell type migrating into the nerve bridge from both nerve ends (Kucenas, 2015; Lewis & Kucenas, 2014). Perineurial cells migrate along the fibrin deposit and form a circumferential tube to control the trajectory of migration of both nerve fibroblasts and endothelial cells (Martini et al., 1990; Schroder et al., 1993; Weis et al., 1994).

At Day 3 postinjury, the number of endothelial cells shows a significant increase to 20% in the nerve bridge due to the dramatic influx of endothelial cells from both nerve ends toward the middle of the bridge. Neutrophils also show a slight increase to 26%, while the ratio of macrophages decreased to 33% and the ratio of fibroblasts decreased to 10%, showing a dynamic and changing cell composition of the nerve bridge (Cattin et al., 2015). Recently, an mRNA sequencing study revealed that macrophages within the nerve bridge show a complex phenotype, which is temporally regulated by the nerve bridge microenvironment (Tomlinson, Zygelyte, Grenier, Edwards, & Cheetham, 2018). We also showed that macrophages in the outermost layer of the nerve bridge have both a different morphology and gene expression profile compared to macrophages localized in the center of the nerve bridge (Dun et al., 2019). Using single-cell transcriptomics, Ydens et al. demonstrated that macrophages in the injured mouse sciatic nerve express a unique transcriptional profile compared with macrophages in other mouse tissues (Ydens et al., 2020). Endothelial cells from both nerve ends are attracted by vascular endothelial growth factor-A (VEGF-A) secreted from macrophages in the nerve gap, they migrate into the nerve gap and form new blood vessels (Cattin et al., 2015).

The majority of newly formed blood vessels are orientated longitudinally in the nerve bridge (Cattin et al., 2015; Schroder et al., 1993; Weis et al., 1994) and they serve as a substrate for subsequent Schwann cell migration (Cattin et al., 2015). Schwann cells from both proximal and

distal nerve stumps migrating toward the nerve bridge can be easily observed passing both nerve stumps on Day 4 postinjury, and they form Schwann cell cords within the nerve bridge by Day 7 (Chen et al., 2019; Torigoe, Tanaka, Takahashi, Awaya, & Hashimoto, 1996). Slit3-Robo1 signaling between Slit3-expressing macrophages and Robo1-expressing migrating Schwann cells in the nerve bridge is required to keep Schwann cells inside the nerve bridge (Dun et al., 2019). EphrinB2-EphB signaling between migrating nerve fibroblasts and Schwann cells is important for Schwann cell sorting and Schwann cell cord formation in the nerve bridge (Parrinello et al., 2010). For a further review of the molecular mechanism of classic axonal guidance signaling pathways and their potential roles in regulating the nerve bridge formation, please see our recent review (Dun & Parkinson, 2020). Regenerating axons are known to follow Schwann cell cords and navigate across the nerve bridge (Cattin et al., 2015; Chen et al., 2019; Dun et al., 2019; Parrinello et al., 2010). For a 2 mm nerve gap, axons begin to enter the distal nerve stump around Day 9 following a transection injury (Chen et al., 2019).

Thus, macrophages, neutrophils, perineurial cells, nerve fibroblasts, endothelial cells, and Schwann cells are the major cell types forming the nerve bridge and they regulate each other's recruitment, migration, and organization in the nerve bridge during regeneration (Cattin et al., 2015; Dun et al., 2019; Kucenas, 2015; Parrinello et al., 2010; Weis et al., 1994; Williams et al., 1983). The formation of the nerve bridge requires an initial fibrin deposit and then involves the coordinated migration of multiple cell types. The cellular make-up and extracellular matrix composition of the nerve bridge changes over time following injury, but as regeneration proceeds the nerve bridge will be replaced largely by Schwann cells and axons, and in time will have a somewhat similar tissue structure to the repaired distal nerve stump.

2.3 | The study of nerve bridge formation using artificial nerve conduits

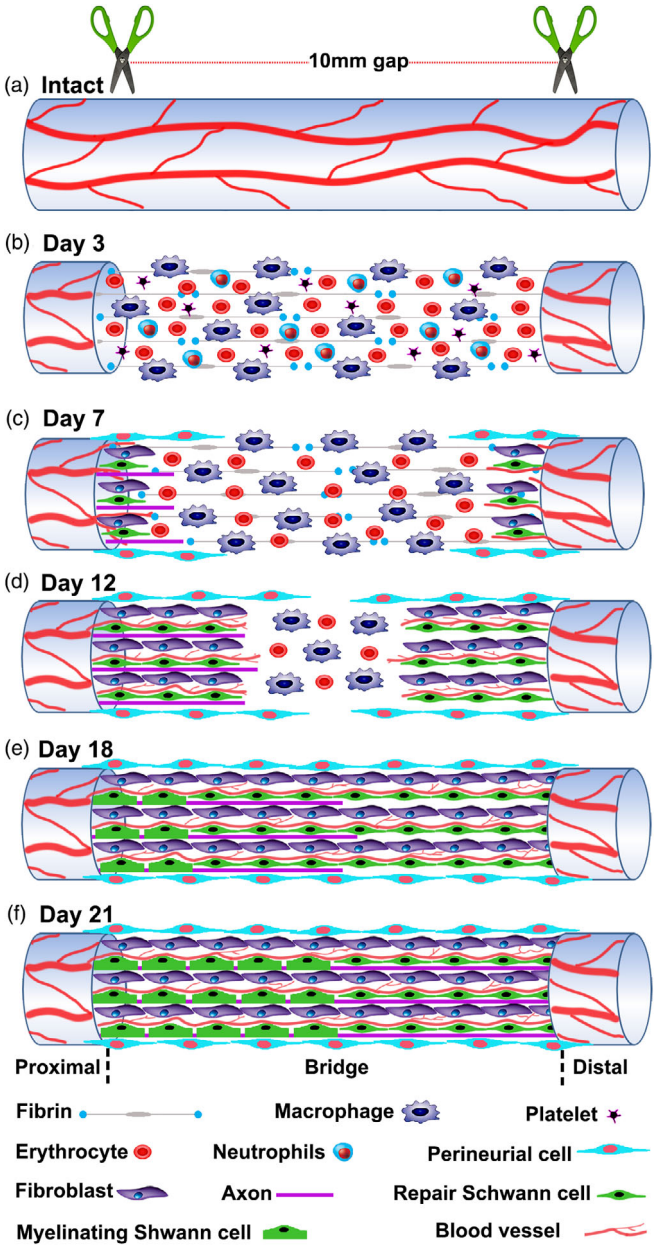
The clinical challenge in the repair of larger peripheral nerve gaps requires the use of biodegradable nerve guidance conduits and several have been developed to repair such nerve gaps of between 5 and 10 mm (Deumens et al., 2010; Kehoe, Zhang, & Boyd, 2012; Ray & Mackinnon, 2010). Therefore, understanding the time course of nerve bridge formation and axon regeneration in nerve guidance conduits will improve the design and the use of nerve guidance conduits in the clinic. In order to study nerve bridge formation in vivo and axon regeneration over longer nerve gaps, Lundborg et al. developed an injury research model by connecting the severed nerve ends using silicone tubing (Lundborg, Dahlin, et al., 1982; Lundborg, Gelberman, Longo, Powell, & Varon, 1982). They showed that the nerve bridge could still form within such a hollow silicone tube for a rat sciatic nerve gap up to 10 mm in length (Lundborg, Dahlin, et al., 1982). The spatial–temporal progress of cellular events of nerve bridge formation within the silicone tube have been studied in some detail with such a 10 mm rat sciatic nerve gap (Figure 2) (Williams et al., 1983).

Within 1 day of surgery, the silicone conduit tube was filled with fluid originating from both nerve ends (Belkas et al., 2004; Lundborg, Longo, & Varon, 1982). Using in vitro neuron cultures, the fluid within the silicone tube was shown to contain neurotrophic factors and extracellular matrix and to stimulate axon growth (Lundborg, Longo, & Varon, 1982). Three days after surgery, a fibrin cord could be observed inside the silicone tubes connecting both nerve ends and the fibrin cord contained numerous erythrocytes, granulocytes, thrombocytes, and macrophages (Schroder et al., 1993; Weis et al., 1994; Williams et al., 1983). The fibrin cord had a parallel and longitudinal arrangement to the nerve and provided a substrate for cells from both nerve ends migrating into the nerve bridge (Williams et al., 1983). As for formation of the bridge without a conduit, perineurial cells were the first cell type from both nerve ends to migrate along the fibrin cord and could be easily observed at Day 7 with an elongated cell shape (Schroder et al., 1993; Weis et al., 1994). Migrating perineurial cells formed a perineurial cell tube surrounding the fibrin and this tube almost bridged the 10 mm gap at Day 12 following transection and repair with the silicone conduit (Figure 2). A continuous tube of perineurial cells connecting the proximal and distal nerve stumps could still be observed at 18 days following surgery. Nerve fibroblasts, endothelial cells, and Schwann cells from both nerve stumps were found to migrate within the perineurial tube toward the middle of the nerve bridge. They migrate at a distance of up to 1 mm from both nerve stumps at Day 7 and almost reach the middle of the nerve bridge at Day 12 (Figure 2). Migrating nerve fibroblasts, endothelial cells, and Schwann cells filled the whole nerve gap within the perineurial tube and formed a continuous cellular cord by Day 18 following surgical repair (Lundborg, Dahlin, et al., 1982; Scaravilli, 1984). Regenerating axons enter the distal nerve stump on Day 21 following surgical repair in this model (Weis et al., 1994).

3 | TRANSGENIC MOUSE MODELS EXPRESSING FLUORESCENT PROTEINS IN SCHWANN CELLS, USEFUL TOOLS TO STUDY SCHWANN CELL MIGRATION IN VIVO

The use of transgenic mouse models expressing fluorescent proteins in Schwann cells such as PLP-GFP (Mallon et al., 2002), S100-GFP (Zuo et al., 2004), S100-RFP (Hirrlinger et al., 2005), and Sox10-Venus (Hirrlinger et al., 2005) have allowed the detailed analysis of Schwann cell behavior and migration following nerve injury. The S100-GFP mouse model was the first mouse model to be used to study Schwann cell migration in vivo after sciatic nerve transection (Hayashi et al., 2007; Tomita et al., 2009; Whitlock et al., 2010). In S100-GFP mice, Schwann cells are labeled with GFP under the control of transcriptional regulatory sequences of the human S100B gene. Although Schwann cells are labeled with bright GFP, studies have shown that a few macrophages also express GFP in this transgenic mouse line (Zuo et al., 2004). More recently, the PLP-GFP mice have been used to visualize in vivo Schwann cell migration (Carr, Parkinson, & Dun, 2017; Cattin et al., 2015; Dun et al., 2019; Stierli et al., 2018). Originally created to label oligodendrocytes in the CNS, with GFP expression driven by the mouse myelin PLP gene promoter, the PLP-GFP mice express

cytoplasmic GFP in both myelinating and nonmyelinating Schwann cells of the peripheral nerves (Mallon et al., 2002). Myelinating Schwann cells and nonmyelinating Schwann cells in the intact nerve are also distinguishable in adult PLP-GFP mice due to a much higher apparent level of GFP expression in nonmyelinating Schwann cells and the recognizable morphology of Remak bundles (Carr et al., 2017; Stierli et al., 2018). Transgenic mice expressing Schwann cell specific Cre-recombinase have also been used to label Schwann cells by crossing with different fluorescence protein reporter lines such as R26R-YFP, R26R-tdTomato, and R26R-Confetti (Benito et al., 2017; Clements et al., 2017; Feltri et al., 1999; Leone et al., 2003; Livet et al., 2007; Madisen et al., 2010; Srinivas et al., 2001; Stierli et al., 2018). These mouse models allow researchers to study the basic behavior and the molecular mechanism of Schwann cell migration in vivo within the nerve bridge following mouse sciatic nerve transection injury.



4 | MIGRATING SCHWANN CELLS DIRECT AXON REGENERATION IN THE NERVE BRIDGE

Accumulating evidence indicates that regenerating axons are unable to cross the nerve gap without Schwann cell guidance (Torigoe et al., 1996, Parrinello et al., 2010, Webber et al., 2011, Rosenberg et al., 2014, Cattin et al., 2015, Dun & Parkinson, 2015). In the zebra fish motor axon injury model, generating just a 9 μm nerve gap by laser transection, regenerating axons lost their directionality and travelled along ectopic trajectories in the nerve bridge when Schwann cells were genetically ablated (Rosenberg et al., 2014). In Sox2, Slit3, and Robo1 knockout mice, Schwann cells migrated aberrantly after sciatic nerve transection injury and regenerating axons followed the path of ectopic migrating Schwann cells leaving the nerve bridge (Dun et al., 2019). More recently in the PLP-GFP mice, we revealed that an early regrowing population of regenerating axons is unable to cross the mouse sciatic nerve gap due to the lack of Schwann cell guidance at their front (Chen et al., 2019). These findings have shown that Schwann cells are the key cell type in the nerve bridge to direct regenerating axons into the distal nerve stump.

4.1 | Misdirected axon regeneration without Schwann cell guidance

It is well known that adult neurons of the peripheral nerves retain the capacity to regenerate (Chen, Yu, & Strickland, 2007; Jessen &

FIGURE 2 Schematic of nerve bridge formation and axon regeneration as seen in a 10 mm rat sciatic nerve gap repaired with a hollow nerve conduit. (a) Rat sciatic nerve before injury. (b) At Day 3 following surgery and silicone tube repair, the gap between the proximal and distal nerve stumps was bridged by a meshwork of fibrin deposit. This fibrin meshwork contains many erythrocytes, neutrophils, platelets, and macrophages. (c) At Day 7, the nerve bridge still contains fibrin deposits, macrophages, and degenerating erythrocytes. Perineurial cells from both nerve ends migrate along the fibrin deposit and form circumferential tubes extending 2 mm from each nerve stump at both ends of the nerve bridge. Inside the perineurial cell tube, fibroblasts, endothelial cells, and Schwann cells from both nerve ends have migrated approximately 1 mm into the nerve bridge. Newly formed capillaries are mainly oriented longitudinally. Very few axons could be observed in the proximal nerve stump and they are not myelinated. (d) At Day 12, migrating perineurial cells have not yet met in the middle of the nerve bridge with a gap distance about 0.5 mm between proximal and distal migrating perineurial cells. There are numerous fibroblasts, Schwann cells, and blood vessels inside the perineurial cell tube. More axons have grown out of the proximal stump and they are associated with Schwann cells but not yet myelinated. Blood vessels start to grow ahead of fibroblasts and Schwann cells. (e) At Day 18, a continuous perineurial cell tube is formed surrounding the nerve bridge. Schwann cells, fibroblasts, and blood vessels also meet in the middle of the nerve bridge. Axons reach the midpoint of the nerve bridge, and are thinly remyelinated. (f) At Day 21 postinjury, axons had reached the distal nerve stump and Schwann cell remyelination is seen up to the midpoint of the bridge

Mirsky, 2016) and they rapidly activate a remarkable intrinsic program to regenerate in response to injury-induced retrograde signals (Rishal & Fainzilber, 2010). Torigoe et al. used the film model and studied the initiation of axon regeneration. In this film model, the transected mouse proximal peroneal nerve was sandwiched between two thin plastic fluorine resin films, resulting in a very thin layer of regenerating tissue being formed between two films; therefore, tissue sectioning was not needed for subsequent analysis in this model. Using this method, they analyzed axon regeneration on the film in the early phase of regeneration up to 6 days. By transmission electron microscopy, they showed that neurite sprouting could be observed at only 3 hr following axotomy from the nodes of Ranvier adjacent to the axotomized nerve stump. Neurites extended and passed through the proximal nerve stump tip and onto the film in just 6 hr (Torigoe et al., 1996). The early time points of neurite sprouting observed by Torigoe et al. were in agreement with Sjöberg and Kanje's observation using an electrophysiological technique (Sjoberg & Kanje, 1990). Using a live cell imaging system in the zebra fish to study GFP-expressing motor axon regeneration, Isaacman-Beck et al. also observed that regenerating axons have extended into the nerve gap at just 7 hr following axotomy (Isaacman-Beck, Schneider, Franzini-Armstrong, & Granato, 2015). Thus, axon regeneration could be easily observed well within the first day after peripheral nerve transection injury.

Following a sciatic nerve transection injury in rodents, Schwann cells within the distal end of the proximal nerve stump and Schwann cells within the proximal end of the distal nerve stump undergo a process of dedifferentiation which takes 2–3 days to complete (Jessen & Mirsky, 2016; Shin et al., 2017; Wang, Teng, & Huang, 2012). After dedifferentiation they can proliferate and migrate, therefore, the start of Schwann cell migration is 2–3 days later than the start of axon extension in the proximal nerve stump (Figure 3) (Chen et al., 2019; Torigoe et al., 1996). Using the film model, Torigoe et al. analyzed Schwann cell migration on the film by S100 antibody staining. They showed that there were no Schwann cells associated with regenerating axons at Day 2 following injury and migrating Schwann cells start to appear near the proximal nerve stump on Day 3. The number of migrating Schwann cells in the proximal nerve stump gradually increased on Day 4 and Schwann cells began to migrate in front of

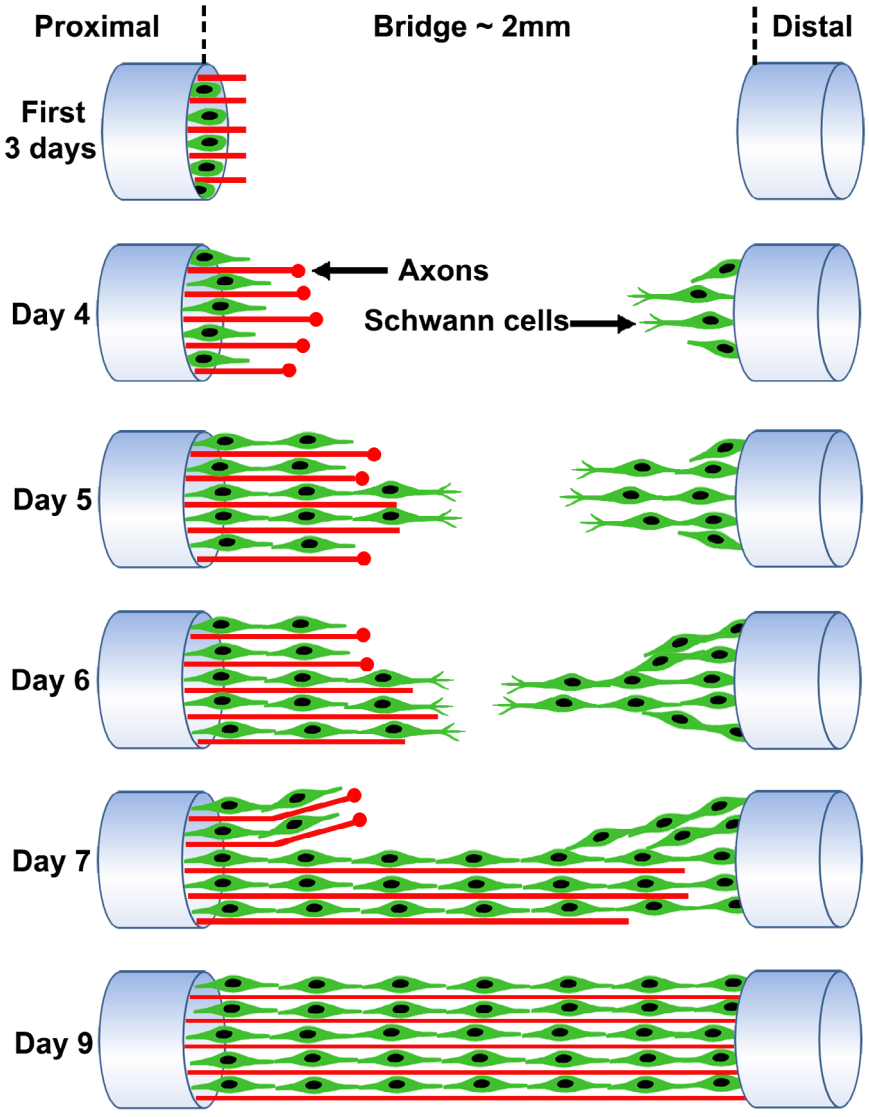


FIGURE 3 Schematic showing Schwann cell-axon interactions in the mouse sciatic nerve bridge at different stages of regeneration. Axons regenerate much earlier than the start of Schwann cell migration following mouse sciatic nerve transection injury. Axons form bundles and often have a ball shape at the regeneration front when there are no Schwann cells localizing ahead of the regenerating axons front before Day 5. Schwann cells migrate in front of axons from Day 5 onward and form Schwann cell cords at Day 7. Leading Schwann cells have two or three leading processes before Day 7. Regenerating axons use Schwann cell cords as a substrate and navigate across the nerve bridge and most axons enter into the distal nerve stump on Day 9

regenerating axons front on Day 5 (Torigoe et al., 1996). However, the nerve bridge was unable to form using the film model; therefore, this research model cannot fully mimic the in vivo nerve bridge microenvironment for Schwann cell migration and axon regeneration. Using PLP-GFP mice, we have studied the detail of Schwann cell migration and axon extension in the nerve bridge in the sciatic nerve (Chen et al., 2019). We also found that regenerating axons regrow in front of the GFP-positive migrating Schwann cells in the nerve bridge for the first 5 days following mouse sciatic nerve transection injury (Chen et al., 2019). Therefore, for the first 5 days following transection injury, axons regenerate into the nerve bridge tissue without Schwann cell guidance at their front (Figure 3).

Torigoe et al. showed that regenerating axons fasciculated with each other and formed axon bundles where there were no migrating Schwann cells at the regeneration front. They termed these regenerating axons as "naked axons" at this stage of regeneration (Torigoe et al., 1996). Using the whole nerve neurofilament antibody staining technique, we also showed that groups of 5–6 regenerating axons formed bundles in the proximal nerve stump on Day 4 and a ball shape often forms at the tips of such axon bundles (Chen et al., 2019; Dun & Parkinson, 2015). The ball shape has been clearly illustrated by Ramón y Cajal with his drawing of regenerating axons in the nerve bridge (Lobato, 2008). This is a typical shape for stalled growth cones due to the lack of a substrate for elongation (Dun et al., 2012; Lobato, 2008). In the PLP-GFP mice, we also observed that some single axons could extend into the nerve bridge on Day 4 when there were no Schwann cells at the front, but such regenerating axons appeared to lack directionality and were not growing toward the distal nerve stump (Chen et al., 2019). The growth cone morphology and lack of directionality of regenerating axons in the nerve bridge at this early stage of regeneration indicate that regenerating axons require Schwann cells at their front to permit the correct trajectory of axon growth.

4.2 | Migrating Schwann cell direct regenerating axons across the nerve bridge

Although Schwann cell migration starts later than axon extension in the proximal nerve stump, Schwann cells overtake regenerating axons on Day 5 and thereafter provide a pathway for regenerating axons navigating across the nerve bridge (Cattin et al., 2015; Dun et al., 2019; Dun & Parkinson, 2015; Parrinello et al., 2010). Both Torigoe's and our studies showed that migrating Schwann cells start to proceed in front of regenerating axons in the proximal nerve stump from Day 5 onward following injury (Chen et al., 2019; Torigoe et al., 1996). Following the pioneer Schwann cells, other Schwann cells attach to each other and form a chain to migrate toward the middle of the nerve bridge from both nerve stumps (Chen et al., 2019). Schwann cell migration in the nerve bridge appears as a classic example of cell chain migration (Aigouy, Lepelletier, & Giangrande, 2008; Dun & Parkinson, 2015; Parrinello et al., 2010). This chain migration is important for the correct Schwann cell cord formation in the nerve bridge (Figure 3). Interestingly, Schwann cells from both nerve ends form chains and migrate toward the middle of the nerve bridge. However, it is currently not known if the migration stops once Schwann cell chains from both nerve ends meet in the middle of the nerve bridge or they continue to migrate toward the opposite nerve ends.

On Day 6, when there are many migrating Schwann cells localizing ahead of the axon front, individual axons emerge from axon bundles and follow migrating Schwann cells across the nerve bridge (Chen et al., 2019). Thus, regenerating axons are directed into the distal nerve stump by Schwann cell cords formed by migrating Schwann cells. Similar to this regeneration process of adult Schwann cells determining the directionality of axon elongation, during embryonic PNS development, about 80% of the nerve front surface is covered by Schwann cell precursors. It is the Schwann cell precursors that are most exposed to the extracellular environment and they form a substrate for advancing growth cones (Wanner, Guerra, et al., 2006; Wanner, Mahoney, et al., 2006).

Both the film model study and our observations in PLP-GFP mice showed that the speed of axon extension has two phases, an initial slow phase (86 μm/day) when there are unaccompanied axons at the front, followed by a faster phase (433 μm/day) when Schwann cells migrate together with axons. The appearance of migrating Schwann cells into the regeneration front coincides with the onset of the second phase of axon growth; therefore, migrating Schwann cells are responsible for the acceleration of axonal growth in the second phase (Torigoe et al., 1996). These observations showed that migrating Schwann cells not only direct axons regenerating toward the distal nerve stump but also increase the speed of axon extension.

5 | IDENTIFYING THE SUBSTRATE FOR SCHWANN CELL MIGRATION IN THE NERVE BRIDGE

5.1 | Fibrin deposit, a possible substrate for Schwann cell migration

Since Williams et al. first showed that a fibrin cord can serve as a substrate for cells from both nerve stumps migrating into the bridge (Williams et al., 1983), fibrin has been considered as the likely substrate for Schwann cell migration into the nerve bridge (Belkas et al., 2004; Daly, Yao, Zeugolis, Windebank, & Pandit, 2012; Deumens et al., 2010). Later Weis et al. showed that the diameter of the fibrin cord within the 10 mm silicone tube was reduced between Days 3 and 7, and in some of the silicone tube samples, the fibrin cord had already mostly disappeared by Day 7 (Schroder et al., 1993; Weis et al., 1994). However, at Day 7, Schwann cells have only migrated approximately 1 mm away from both nerve stumps within the 10 mm silicone tubes (Williams et al., 1983), indicating that migrating Schwann cells must find a new substrate for migration in order to bridge the rest of the 8 mm gap between the advancing cell fronts. Furthermore, in vitro Schwann cell migration assays on mixed fibrin/fibronectin substrates demonstrated that increasing the amount of fibrin in the substrates inhibited Schwann cell migration (Akassoglou, Akpinar, Murray, & Strickland, 2003).

Although a positive effect for peripheral nerve gap repair has been seen using fibrin matrices to deliver growth factors into nerve guidance conduits, these observed positive effects may have rather resulted from the use of growth factors in the fibrin matrices (Wood, Moore, et al., 2009; Wood, Borschel, & Sakiyama-Elbert, 2009). Therefore, it remains to be examined in vivo to what extent Schwann cells migrate along a fibrin substrate.

5.2 | Schwann cells migrate along the regenerating axons in the proximal nerve stump at early stages of regeneration

Using the film model, Torigoe et al. analyzed Schwann cell migration on the film and showed that proximal Schwann cells preferred axonal surfaces over any other environmental structure as a substrate for migration (Torigoe et al., 1996). Combining our whole sciatic nerve neurofilament staining technique with use of the PLP-GFP mice, we also showed that Schwann cells use axons as a substrate to migrate upon in the proximal nerve stump before Day 5 (Chen et al., 2019). During PNS development, Schwann cell precursors migrate as a continuous chain along the developing nerves (Sepp & Auld, 2003; Sepp, Schulte, & Auld, 2000), and N-cadherin expression in Schwann cell precursors has been proposed as the primary reason that Schwann cell precursors migrate along the developing nerves (Wanner, Guerra, et al., 2006). Following injury, Schwann cells upregulate N-cadherin expression (Parrinello et al., 2010; Roberts et al., 2017), and in vitro coculture of adult rat Schwann cells with embryonic dorsal root ganglion neurons revealed that N-cadherin mediates Schwann cell alignment along axons (Wanner & Wood, 2002). Thus, the expression of adhesion molecules such as N-cadherin on Schwann cells could be one reason that Schwann cells in the proximal nerve stump initially use regenerating axons as a substrate to migrate before Day 5 (Figure 4). However, Schwann cells have to find new substrate to migrate upon from Day 5 onward once they proceed in front of regenerating axons. Furthermore, there are no axons acting as substrates for Schwann cell migration from the distal nerve stump, indicating that Schwann cells require a different type of substrate upon which to migrate into the nerve bridge.

5.3 | Newly formed blood vessels as a substrate for guiding Schwann cell migration and cord formation

Schroder et al. observed that Schwann cells concentrated around the outgrowing blood vessels in the silicone tubes (Schroder et al., 1993),

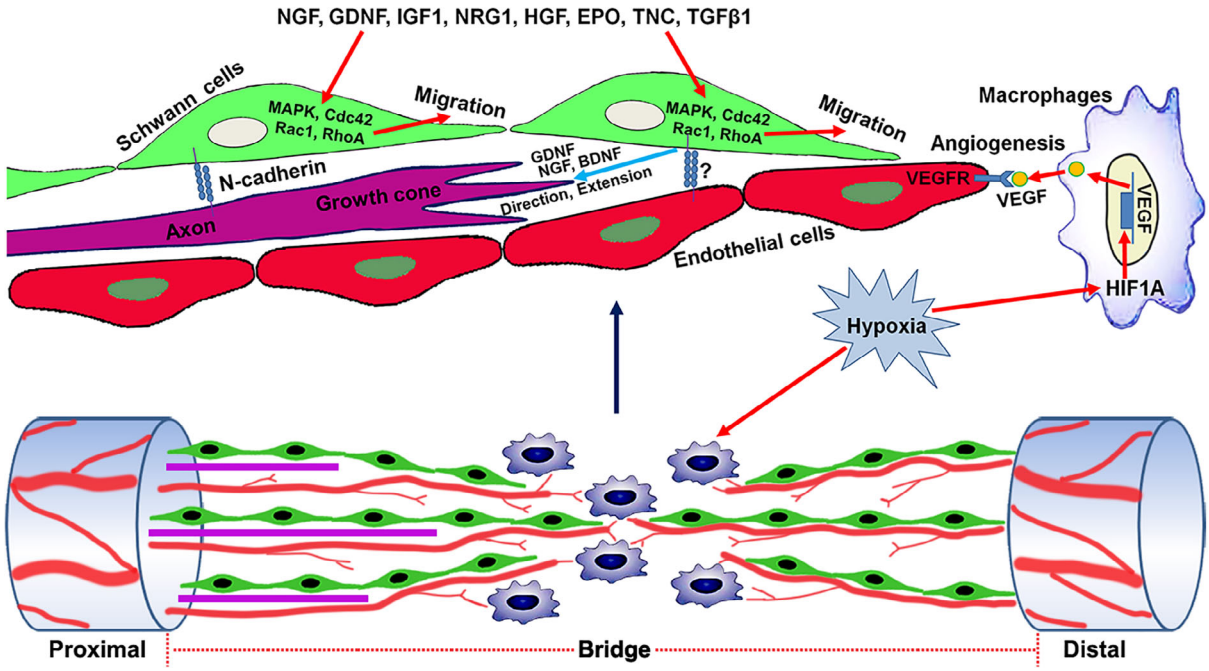


FIGURE 4 Signals activating Schwann cell migration and substrates for Schwann cell migration in the nerve bridge. Upon nerve transection, macrophages in the nerve bridge sense hypoxia and upregulate vascular endothelial growth factor (VEGF) to induce blood vessel regeneration. The Schwann cell intrinsic migration property is activated by a number of growth factors illustrated. Upon putative activation of mitogen-activated protein kinase (MAPK), Cdc42, Rac1, and RhoA signaling, Schwann cells at both nerve ends actively search for a substrate and migrate. In the proximal nerve stump, Schwann cells initially use regenerating axons as a substrate to migrate, but then use blood vessels as an alternative substrate once they overtake elongating axons. Schwann cells in the distal nerve stump also use blood vessels as substrate to migrate. Migrating Schwann cells from both nerve ends meet in the middle of the nerve bridge and form Schwann cells cords to direct axon regeneration. Nerve growth factor (NGF), BDNF, and glial cell line derived neurotrophic factor (GDNF) are critical signals that Schwann cells utilize to direct axons into the distal nerve stump

which was later investigated by Cattin et al. to show that Schwann cells use newly regenerated blood vessels in the nerve bridge as a substrate for migration (Cattin et al., 2015). In their studies, Cattin et al. first showed that blood vessels emanate from both proximal and distal stumps after sciatic nerve transection injury in mouse and rat, and the nerve bridge is fully vascularized prior to the start of Schwann cell migration. Around 80% of newly regenerated blood vessels in the nerve bridge were orientated in the same direction as subsequent Schwann cell migration. Immunostaining of the vasculature in the nerve bridge of PLP-GFP mice showed that migrating Schwann cells closely associated with the blood vessels in the bridge. Schwann cells frequently make direct physical contacts with blood vessels via long protrusions (Cattin et al., 2015). Moreover, it was demonstrated in vitro that Schwann cells are able to migrate along capillary-like endothelial cells using an in vitro system (Cattin et al., 2015).

Cattin et al. further showed that most macrophages (>80%) sense the hypoxic environment in the nerve bridge and increase Hypoxia-Inducible Factor 1-alpha (HIF-1a) expression. Subsequently, HIF-1a drives VEGF-A expression in macrophages (Figure 4). Using a Transwell assay, they demonstrated that endothelial cells migrate in response to VEGF-A but not Schwann cells. To address whether VEGF-A induced blood vessel formation was sufficient to direct Schwann cell migration in vivo, Cattin et al. implanted heparin beads loaded with recombinant human VEGF165 into the nerve gap in vivo to attempt to misdirect blood vessel regeneration. Six days after VEGF-A bead implantation, they found that Schwann cells followed the misdirected regenerating blood vessels and migrated away from the nerve bridge. Thus, Cattin et al. confirmed that the regenerating blood vessels are the most important substrate in the nerve bridge for Schwann cell migration (Cattin et al., 2015).

6 | THE INTRINSIC MIGRATORY PROPERTY OF SCHWANN CELLS

Current research evidence suggests that the intrinsic migratory properties of Schwann cells are activated during the processes of dedifferentiation, and they then actively search for a substrate and begin to migrate. The intrinsic migratory properties of Schwann cells have been studied by Wang et al. using a single-cell migration assay with cultured rat sciatic nerve explants (Wang et al., 2012), which effectively mimics the in vivo Schwann cell response to injury (Shin et al., 2017). Wang et al. showed that Schwann cells could spontaneously migrate away from nerve explants onto laminin-coated coverslips after 48–72 hr of culture. Migrating Schwann cells from sciatic nerve explants display high motility with a majority of them showing a bipolar morphology. Gatto et al. showed that asymmetric ERM (ezrin, radixin, moesin) protein activation in retracting cell processes was required to maintain a bipolar shape in Schwann cells during migration (Gatto, Walker, & Lambert, 2003, 2007). To migrate out from explants onto laminin-coated coverslips, dedifferentiated Schwann cells first sent out long processes and then the nucleus moved forward within the processes to the front end of the cell. The back part of the cell processes then retracted and the front processes extended once more (Wang et al., 2012). We recently showed in the PLP-GFP mice that leading migratory Schwann cells in the nerve bridge have two or three leading processes, suggesting that they are responsible for detecting migrating substrate and/or guidance cues (Chen et al., 2019).

7 | THE ACTIVATION OF SMALL GTPASES AND MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAYS FOR SCHWANN CELL MIGRATION

7.1 | The activation of small GTPases for Schwann cell migration

Signaling pathway analysis of RNA sequencing data from rat distal sciatic nerve revealed that the activation of growth factor receptors, G-protein coupled receptors and integrins following injury could possibly activate the Rho family of small GTPases Cdc42, Rac1, and RhoA in Schwann cells (Wang, Shan, Pan, & Yi, 2018). The active forms of Cdc42, Rac1, and RhoA are all key regulators of cytoskeletal dynamics and Schwann cell migration (Feltri, Suter, & Relvas, 2008; Lambrechts, Van Troys, & Ampe, 2004; Yamaguchi & Condeelis, 2007). Activated Cdc42 and Rac1 regulate Schwann cell leading process extension and soma translocation through the control of F-actin polymerization at the front edge of leading process (Wang et al., 2012). Treatment of cultured Schwann cells with cytochalasin D, an F-actin polymerization inhibitor, resulted in the collapse and retraction of leading processes and an inhibition of Schwann cell migration (Wang et al., 2012; Wang, Teng, & Huang, 2013). Injection of cytochalasin D to the injury site also inhibited Schwann cell migration in vivo (Wang et al., 2018).

Application of the RhoA inhibitor Y-27632 at the leading front of migrating Schwann cells reduced soma translocation, indicating that activated RhoA is required to regulate soma translocation during Schwann cell migration (Wang et al., 2012, 2013; Wang et al., 2018). Myosin II has also been identified as a key player in soma translocation in many types of cells and myosin II is known to be activated by RhoA (Conti & Adelstein, 2008; Solecki et al., 2009). Schwann cells lacking myosin II activity exhibit a multipolar morphology and fail to elongate properly along the axon and myelinate during peripheral nerve development (Wang, Tewari, Einheber, Salzer, & Melendez-Vasquez, 2008). Myosin II activity displays a polarized distribution in Schwann cells with the leading processes having higher levels of active myosin II than the soma and trailing processes. Activation of myosin at the front of the Schwann cell leading process accelerated soma translocation. In contrast, inhibiting polarized myosin II activity in the front of leading process not only induced the retraction of leading process but also inhibited soma translocation. Thus, RhoA activation is required to control polarized distribution of myosin II activity in migrating Schwann cells and regulates soma translocation during migration.

7.2 | The activation of mitogen-activated protein kinase signaling pathways for Schwann cell migration

Recent studies have found that Schwann cell migration could be guided by endogenous electric fields (EFs) (McKasson, Huang, & Robinson, 2008; Yao, Li, Knapp, & Smith, 2015). EFs have not only been detected in developing and regenerating tissues (Hotary & Robinson, 1990; McCaig, Rajnicek, Song, & Zhao, 2005) but have also been used in human clinical trials to enhance the repair of spinal cord injuries (Shapiro et al., 2005; Tator, 2005). McKasson et al. showed that Schwann cells are more sensitive to EF-stimulated migration than any other studied cell type. At 100 mV/mm^{-1}, Schwann cells showed a strong movement toward the anode rather than cathode as hippocampal neurons and neural crest cells do (McKasson et al., 2008). Reversal of EF poles reversed the direction of Schwann cell migration (Yao et al., 2015). Further studies showed that EF stimulation promoted the release of NGF and BDNF by Schwann cells, and promoted neurite outgrowth (Huang, Ye, Hu, Lu, & Luo, 2010; Koppes et al., 2014; Koppes, Seggio, & Thompson, 2011). Yao et al. studied the differential gene expression between EF stimulated and unstimulated rat Schwann cells using an RNA-seq approach. Pathway analysis revealed that 10 pathways are upregulated while 21 pathways are downregulated. Among these pathways, mitogen-activated protein kinase (MAPK) pathway is the most significantly upregulated pathway upon EF stimulation, indicating that MAPK pathway is important for the regulation of Schwann cell motility. Indeed, all three canonical MAPK pathways, ERK, p38, and JNK, are rapidly and consistently activated in Schwann cells after peripheral nerve injury (Agthong, Kaewsema, Tanomsridejchai, & Chentanez, 2006; Parkinson et al., 2008; Roberts et al., 2016; Sheu, Kulhanek, & Eckenstein, 2000; Yamazaki et al., 2009; Yang et al., 2012). Knockout of cJun, a downstream target of JNK, prevented Schwann cell morphology change to a migratory bipolar shape and reduced Schwann cell motility (Arthur-Farraj et al., 2012), while overexpressing cJun in Schwann cells increased cell motility (Huang et al., 2015). In vivo, overexpressing cJun in Schwann cells also accelerated axonal regeneration, remyelination and functional recovery after mouse sciatic nerve crush injury (Fazal et al., 2017). The regulation and the activation of ERK, p38, and JNK signaling pathways in Schwann cells requires the activation of membrane receptors such as GPCRs, receptor tyrosine kinases, cytokines receptors, and integrins. Thus, the further study of signaling molecules that could interact with these receptors, in particular signaling molecules that are upregulated in the peripheral nerves following injury, will enable us to understand better the molecular mechanism driving Schwann cell migration.

8 | SIGNALS PROMOTING SCHWANN CELL MIGRATION TOWARD THE MIDDLE OF THE NERVE BRIDGE

To date, the molecular mechanisms regulating Schwann cell migration into the nerve bridge from both nerve ends are still poorly understood. Current understanding about signals that have the ability to promote Schwann cell migration are largely obtained from in vitro studies and reports from studying PNS development (Cornejo et al., 2010; Heermann & Schwab, 2013). During PNS development, Schwann cell precursors derive from the neural crest, migrate along axons, and mature to Schwann cells (Table 1). Using in vitro assays such as wound healing assay and Transwell migration assays on cultured Schwann cells, several signaling molecules including NRG1 Type III, neurotrophins, erythropoietin and glial cell line derived neurotrophic factor (GDNF), have all been identified to promote the motility and migration of Schwann cells along axons (Cornejo et al., 2010; Heermann & Schwab, 2013). Although Schwann cells in the distal nerve stump away from the transection site do not migrate, they are activated and extend their length to serve as a substrate for axon extension (Gomez-Sanchez et al., 2017). In this section, we will review signals that are activated in the distal nerve stump during peripheral nerve regeneration because these signals have the ability to promote in vitro Schwann cell migration and some of them have been demonstrated to promote in vivo Schwann cell migration (Figure 4, Table 1).

8.1 | Roles of neurotrophins, GDNF, and insulin-like growth factor-1

Neurotrophins consist of NGF, BDNF, and neurotrophins 3-5 (NT3-5), and they activate two structurally unrelated receptor types, the Tropomyosin-receptor-kinase (Trk) and the p75 neurotrophin receptor ($p75^{NTR}$). During development, NT3 acts through the TrkC receptor tyrosine kinase on Schwann cells and stimulates Schwann cell migration via Rac1/Cdc42 and c-Jun N-terminal kinase signaling pathways (Yamauchi, Chan, & Shooter, 2003, 2004; Yamauchi, Miyamoto, Tanoue, Shooter, & Chan, 2005). NT3 is downregulated upon the onset of myelination and this downregulation is required for the Schwann cell transition into the myelinating phenotype (Yamauchi et al., 2003, 2004; Yamauchi et al., 2005). The expression of NT3 in the adult peripheral nerves is very low and it is further downregulated in the distal nerve stump following injury (Funakoshi et al., 1993; Santos, Gonzalez-Perez, Navarro, & Del Valle, 2016). Thus, NT3 is unlikely to play a role in Schwann cell migration during peripheral nerve regeneration.

In contrast, BDNF, NGF, GDNF, and insulin-like growth factor-1 (IGF-1) are all upregulated in injured peripheral nerves (Cheng, Steinway, Delaney, et al., 2000; Funakoshi et al., 1993; Heumann et al., 1987; Meyer, Matsuoka, Wetmore, Olson, & Thoenen, 1992; Pu, Zhuang, & Ishii, 1995; Xu et al., 2013). BDNF was found to interact with $p75^{NTR}$ and to inhibit NT3 induced Schwann cell migration during development; BDNF-$p75^{NTR}$ interaction appears to be required to enhance Schwann cell myelination rather than migration (Ng, Chen, Mandemakers, Cosgaya, & Chan, 2007; Yamauchi et al., 2003, 2004; Yamauchi et al., 2005). in vitro migration assays also showed that BDNF has no effect on Schwann cell migration (Maniwa et al., 2003). In contrast, NGF, GDNF, and IGF-1 are all able to promote migration of cultured Schwann cells (Anton et al., 1994; Cao et al., 2007;

TABLE 1 Summary of signals regulating Schwann cell migration

Signals	Receptors	Signaling pathways	In vivo upregulation in model system	Migration assay methods
NGF	$p75^{NTR}$	NFκB (Carter et al., 1996).	Rat sciatic nerve transection (Funakoshi et al., 1993; Heumann, Korsching, Bandtlow, & Thoenen, 1987).	DRG explant culture onto sciatic nerve sections (Anton, Weskamp, Reichardt, & Matthew, 1994), chemotaxis chamber (Maniwa, Iwata, Hirata, & Ochi, 2003), sciatic nerve explant migration assay, inverted coverslip migration assay (Cao et al., 2007), wound healing assay (Cornejo et al., 2010).
GDNF	Ret and GFRα1	ERK, CREB, PKA, PKC	Mouse sciatic nerve crush (Naveilhan, ElShamy, & Ernfors, 1997); Rat sciatic nerve transection (Xu et al., 2013).	Wound healing assay (Cornejo et al., 2010), Transwell migration assay (Cornejo et al., 2010; Yi et al., 2016).
IGF1	IGF1R	PI-3 kinase, Rac1, FAK	Rat sciatic nerve transection (Cheng et al., 1996).	Gold motility assays (Cheng, Steinway, Delaney, Franke, & Feldman, 2000; Cheng, Steinway, Russell, & Feldman, 2000).
Nrg1 Type I	ErbB2 and ErbB3	ERK, PI-3 kinase, Rac1, CDC42, JNK, cjun, Gab2, Shp2, Src, FAK	Rat sciatic nerve transection (Carroll, Miller, Frohnert, Kim, & Corbett, 1997), Mouse sciatic nerve crush (Stassart et al., 2013).	DRG explant culture onto sciatic nerve sections (Mahanthappa, Anton, & Matthew, 1996), wound healing assay (Meintanis, Thomaidou, Jessen, Mirsky, & Matsas, 2001), time-lapse imaging (Foxd3:GFP Zebrafish) (Perlin, Lush, Stephens, Piotrowski, & Talbot, 2011).
HGF	c-met	ERK, CREB, c-fos, AKT, Gab2	Mouse sciatic nerve crush (Ko, Lee, Lee, Nho, & Kim, 2018).	Sciatic nerve explant migration assay (Shin et al., 2017), Transwell migration assay (Ko, Lee, Lee, et al., 2018; Ko, Lee, Nho, & Kim, 2018), in vivo nerve gap migration assay (Shin et al., 2017).
EPO	EpoR	ERK, Jak2	Rat sciatic nerve crush (Li, Gonias, & Campana, 2005).	Transwell migration assay (Inoue et al., 2010).
TNC	β1-integrin	Rac1	Rat sciatic nerve transection (Zhang et al., 2016).	Transwell migration assay, wound healing assay, in vivo nerve gap migration assay (Zhang et al., 2016).
TGFβ1	TGFβR	Smad2/3, ERK, JNK	Rat sciatic nerve transection (Rufer, Flanders, & Unsicker, 1994; Scherer, Kamholz, & Jakowlew, 1993).	In vivo nerve gap migration assay (Clements et al., 2017), wound healing assay (Muscella, Vetrugno, Cossa, & Marsigliante, 2020).

Abbreviations: EPO, erythropoietin; HGF, hepatocyte growth factor; IGF1, insulin-like growth factor 1; GDNF, glial cell line derived neurotrophic factor; NGF, nerve growth factor; Nrg1, neuregulin1; TGFβ1, transforming growth factor β1; TNC, tenascin-C.

Cheng, Steinway, Russell, & Feldman, 2000; Cornejo et al., 2010; Ju et al., 2015; Maniwa et al., 2003), but it remains unclear if they play an in vivo role in activating Schwann cell migration into the nerve bridge following peripheral nerve transection injury.

8.2 | NRG1 Type I signaling

Expression of the soluble isoform of NRG1 Type I is significantly increased in Schwann cells of the distal nerve following sciatic nerve transection. NRG1 Type I mRNA in Schwann cells is undetectable in uninjured nerves, but is strongly induced within 24 hr of injury and remains upregulated until at least 2 weeks following injury (Carroll et al., 1997; Stassart et al., 2013). An increase of NRG1 Type I protein in Schwann cells was detected at 1 day following injury and persisted for at least 4 weeks (Stassart et al., 2013). Mahanthappa et al. showed that secreted NRG1 Type I could increase the motility of denervated Schwann cells (Mahanthappa et al., 1996). Although NRG1 receptors ErbB2 and ErbB3 are expressed in Schwann cells of uninjured nerves, they are upregulated in response to injury, indicating an important role of this signaling pathway in aspects of peripheral nerve regeneration. The upregulation of ErbB2 and ErbB3 showed a similar pattern as the NRG1 Type I upregulation. Sustained ErbB2 and ErbB3 upregulation is seen from 3 days postinjury (Carroll et al., 1997). Guertin et al. showed that ErbB2 and ERK1/2 were strongly activated at 4 days after injury in the distal nerve stump (Guertin, Zhang, Mak,

Alberta, & Kim, 2005), but the activation of ErbB2 and ERK1/2 phosphorylation were absent in Schwann cell specific NRG1 Type I knockout mice, suggesting that the activation of ErbB2 and ERK1/2 is NRG1 Type I dependent (Stassart et al., 2013). Later studies have revealed that several signaling pathways including MAPK, Rac1/Cdc42-JNK, and Shp2-Src-FAK signaling pathways are also involved in NRG1 Type I-induced Schwann cell migration (Grossmann et al., 2009; Mahanthappa et al., 1996; Yamauchi, Miyamoto, Chan, & Tanoue, 2008). These findings indicate that NRG1 Type I could be one of the signals activating the intrinsic migratory properties of Schwann cells.

8.3 | Hepatocyte growth factor signaling

Hepatocyte growth factor (HGF) is a paracrine cellular growth factor and binds to the c-Met receptor. It has a major role in embryonic organ development, cell migration, adult organ regeneration, and wound healing (Imamura & Matsumoto, 2017; Nakamura, Sakai, Nakamura, & Matsumoto, 2011). HGF protein levels are highly increased in fibroblasts of the injured peripheral nerves (Ko, Lee, Lee, et al., 2018; Lee et al., 2017), while the c-Met receptor is upregulated in dedifferentiated Schwann cells (Ko, Lee, Lee, et al., 2018; Shin et al., 2017). Shin et al. found that HGF cooperates with NRG1 and controls Schwann cell migration through the regulation of Grb2-associated binder-2 (Gab2) (Shin et al., 2017). NRG1 treatment upregulates Gab2 expression in cultured Schwann cells and nerve explants through a Rac-JNK-cJun pathway, but the tyrosine phosphorylation of Gab2 is not regulated by NRG1-ErbB2 signaling (Shin et al., 2014; Shin et al., 2017). Screening with receptor specific inhibitors in sciatic nerve explants to identify signals regulating Gab2 phosphorylation, Shin et al. found that crizotinib, a selective c-Met inhibitor, suppressed the tyrosine phosphorylation of Gab2, indicating that HGF regulates Gab2 phosphorylation in Schwann cells (Shin et al., 2017). HGF treatment not only induced tyrosine phosphorylation of Gab2 but also induced c-Met phosphorylation, ERK and AKT activation in Schwann cells (Ko, Lee, Lee, et al., 2018; Ko, Lee, Nho, & Kim, 2018; Shin et al., 2017). HGF treatment also increased the level of cJun expression in Schwann cells and promoted Schwann cell migration (Ko, Lee, Lee, et al., 2018; Ko, Lee, Nho, & Kim, 2018; Shin et al., 2017). Exogenous application of HGF around the injury site enhanced peripheral nerve regeneration while treatment with a c-Met inhibitor (PHA-665752) following injury inhibited peripheral nerve regeneration (Ko, Lee, Lee, et al., 2018). Because both HGF and NRG1 regulate Schwann cell migration and Gab2 activity, the effect of Gab2 upon Schwann cell migration was investigated using Gab2 null mice. In Gab2 null mice after sciatic nerve transection injury, the total number of Schwann cells migrating into the nerve bridge was significantly reduced and the number of regenerating axons entering the distal stump was also significantly reduced (Shin et al., 2017). Schwann cells from Gab2 null mice in culture exhibited shorter bipolar processes and a lack of basal membrane ruffles upon HGF treatment. Thus, their studies confirmed that HGF cooperates with NRG1 Type I and regulates Schwann cell migration into the nerve bridge after peripheral nerve transection injury.

8.4 | Erythropoietin signaling

Inoue et al. showed that erythropoietin (Epo) is also significantly increased in Schwann cells of the distal nerve following injury (Li et al., 2005). The Epo receptor (EpoR) is expressed by Schwann cells in intact peripheral nerves and its expression increased threefold at 7 days postinjury (Li et al., 2005). Epo treatment of injured nerves or cultured Schwann cells induces EpoR tyrosine-phosphorylation, ERK activation, and fibronectin expression. Epo treatment also recruits β1 integrin to the cell surface and promoted Schwann cell migration (Inoue et al., 2010). JAK2, a nonreceptor tyrosine kinase, is also activated in Schwann cells following Epo binding to EpoR. When Schwann cells were pretreated with a JAK2 inhibitor (AG490), Epo-induced β1 integrin relocalization to the cell surface was blocked, suggesting that Epo stimulates Schwann cell migration by a JAK2-dependent mechanism that promotes β1 integrin subunit relocation to the cell surface (Inoue et al., 2010).

8.5 | Tenascin-C and betacellulin signaling

Recent advances in gene expression profiling have enabled researchers to identify other novel signals that are highly upregulated in the distal nerve stump with the ability to promote Schwann cell migration. Tenascins are a highly conserved family of large oligomeric glycoproteins in the ECM of vertebrate organisms (Chiquet-Ehrismann, Kalla, Pearson, Beck, & Chiquet, 1988). Tenascin-C (TNC), one of the four members of the tenascin family, is dramatically upregulated within injured peripheral nerves (Zhang et al., 2016). Gene expression profile and pathway analysis with microarray data from rat distal nerve stump predicted that TNC could regulate cell migration though a β1-integrin-dependent pathway during peripheral nerve regeneration. A subsequent study showed that TNC is highly expressed in fibroblasts within injured peripheral nerves. Co-immunoprecipitation assays demonstrated that TNC could directly bind to β1-integrin on Schwann cells. TNC treatment of rat primary Schwann cells increased Rac1 and RhoA activity and promoted Schwann cell migration. In fibroblast and Schwann cell cocultures, inhibition of TNC expression in fibroblasts suppressed Schwann cell migration. Further studies using Matrigel to deliver exogenous TNC protein in a 5 mm rat sciatic nerve gap showed that exogenous TNC enhanced Schwann cell migration and axonal regrowth in vivo (Zhang et al., 2016). Gene expression profiling also identified another highly upregulated growth factor betacellulin (Btc) in Schwann cells of the distal nerve stump (Vallieres et al., 2017). Btc is a ligand of the ErbB family receptors and Schwann cells in the distal nerve stump are known to express ErbB2 and ErbB3 (Carroll et al., 1997). Schwann cells proliferate more actively in the distal sciatic nerve stump of Btc overexpressing mice while they proliferate less in Btc knockout mice. Btc overexpression was found to accelerate the recovery of sciatic nerve function following injury (Vallieres et al., 2017). In endothelial cells, Btc interacts with ErbB receptors and activates ERK and AKT signaling to promote endothelial cell migration (Kim et al., 2003).

However, the direct effect of Btc upon Schwann cell migration has not yet been measured.

8.6 | Schwann cell partial epithelial-mesenchymal transition and TGF-β1 signaling

Recently, Arthur-Farraj et al. showed that Schwann cells in the distal nerve stump undergo a partial epithelial-mesenchymal transition (EMT) following injury (Arthur-Farraj et al., 2017). EMT represents a process of cellular reprogramming to convert epithelial cells to a motile mesenchymal phenotype (Kim et al., 2018). RNA-seq analysis has revealed the enrichment of mRNAs and miRNAs for an EMT phenotype in mouse distal nerve samples. This involved a downregulation of RNAs associated with EMT including E-cadherin, Wt1, Fgf1, Ndrg1, mir30, mir33, and mir137, and upregulation of EMT associated RNAs including Tgfβ1, Met, Hmga2, mir21, mir221, and mir222 (Arthur-Farraj et al., 2017). EMT has been intensively studied in development and cancer, and is now known to be controlled by multiple signals including secreted signals such as the TGFβ1 family proteins. Following peripheral nerve injury, TGF-β1 is upregulated in Schwann cells, endoneurial fibroblasts, and infiltrating macrophages (Kiefer, Streit, Toyka, Kreutzberg, & Hartung, 1995; Parkinson et al., 2001; Rufer et al., 1994; Scherer et al., 1993; Sulaiman & Nguyen, 2016). in vitro TGFβ1 treatment on RSC96 Schwann cells promoted Schwann cell migration (Muscella et al., 2020) and TGF-β1-treated Schwann cells are more effective in promoting in vivo axonal regeneration (Sulaiman & Nguyen, 2016). Clements et al. found that EMT signatures are more prominent in Schwann cells in the bridge than Schwann cells in the distal nerve stump, because the wound microenvironment in the nerve bridge increases Schwann cell EMT characteristics (Clements et al., 2017). Clements et al. also demonstrated that TGFβ1 signaling, localized in the wound microenvironment, increased the Schwann cell EMT phenotype. They studied Schwann cell migration in Schwann cell specific TGF-β receptor II knockout mice and found that Schwann cell migration was reduced in these mice (Clements et al., 2017). Therefore, it appears that TGFβ1 is also a key mediator of Schwann cell migration within the nerve bridge.

9 | SIGNALS THAT SCHWANN CELLS UTILISE TO DIRECT AXONS ACROSS THE NERVE BRIDGE

Dedifferentiated Schwann cells upregulate growth factors and neurotrophins such as NGF, BDNF, GDNF, VEGF, neurotrophin 4, glial growth factor, ciliary neurotrophic factor, fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), and IGFs (Gordon, 2009; Jessen & Mirsky, 2016; Madduri & Gander, 2012). Using a variety of approaches including Schwann cell or stem cell overexpression, viral infection, nanoparticles, hydrogel, or Matrigel, these growth factors and neurotrophins have been delivered into injured peripheral nerves and their effects upon promoting axon regeneration have been extensively examined (Hoyng et al., 2014; Madduri & Gander, 2012). Among all the investigated growth factors and neurotrophins, NGF, BDNF, and GDNF overexpression caused the trapping of regenerating axons and the formation of axon coils (Eggers et al., 2013; Hoyng et al., 2014; Santosa et al., 2013; Tannemaat et al., 2008). NGF overexpression selectively trapped sensory axons and impaired sensory functional recovery while BDNF overexpression selectively trapped motor axons and impaired motor functional recovery. GDNF overexpression predominantly trapped motor axons and impaired motor functional recovery (Hoyng et al., 2014). The outcome of axon entrapment has been considered as the result of NGF, BDNF, and GDNF's chemoattractant properties for axons (Dudanova, Gatto, & Klein, 2010; Tannemaat et al., 2008). Certainly, any therapeutic use of these neurotrophins will require a graded or strictly controlled time course of use, but these observations indicated that migrating Schwann cells might utilize NGF, BDNF, and GDNF as cues to direct axon regeneration in the nerve bridge (Figure 4).

NGF is specifically upregulated in Schwann cells from sensory nerves, whereas the expression of BDNF is more pronounced in Schwann cells from motor nerves (Hoke et al., 2006). NGF exclusively promoted sensory axon regeneration while BDNF selectively promoted motor axon regeneration (Santos et al., 2016). The NGF and BDNF selective effect on sensory or motor axons has been explained as the selective expression pattern of their high affinity receptors. Sensory neurons express the NGF high affinity receptor tropomyosin receptor kinase A whereas motor neurons express the BDNF high affinity receptor TrkB (Averill, McMahon, Clary, Reichardt, & Priestley, 1995; Tovar, Ramirez-Jarquin, Lazo-Gomez, & Tapia, 2014). Although NGF appears to be necessary and beneficial for sensory nerve regeneration, it has been shown to participate in the development of thermal and mechanical hyperalgesia (Barker, Mantyh, Arendt-Nielsen, Viktrup, & Tive, 2020; Hu et al., 2017). Therefore, the use of NGF for peripheral nerve regeneration should also be considered in pain development following use of NGF to repair the nerve. Sciatic nerve grafting from Schwann cell specific BDNF knockout mice into wild type mice showed that axon regeneration into Schwann cell specific BDNF knockout nerves was markedly reduced (Wilhelm et al., 2012). In contrast, GDNF expression is upregulated in both myelinating and nonmyelinating Schwann cells (Naveilhan et al., 1997; Trupp et al., 1995; Xu et al., 2013). GDNF upregulation is found to be mediated by the activation of Schwann cell purinergic receptors followed by protein kinase C and D activation to increase GDNF transcription (Xu et al., 2013). GDNF typically signals via its receptor GFRα1 complexed with RET-tyrosine kinase, and utilizes MAPK and phosphatidylinositol 3-kinase/AKT signaling pathways to execute its biological effects (Takahashi, 2001). Although both sensory and motor neurons express GFRa1 and Ret (Keast, Forrest, & Osborne, 2010; Naveilhan et al., 1997), GDNF has a much stronger ability to promote motor axon regeneration (Hoyng et al., 2014; Tannemaat et al., 2008). Peripheral nerve regeneration is dramatically impeded in conditional GDNF knockout mice after crush injury (Dong et al., 2018). However, how migrating Schwann cells utilizing GDNF as a guidance cue for regenerating axons crossing the nerve bridge has not been studied in conditional GDNF knockout mice following transection injury.

10 | FUTURE DIRECTIONS

Peripheral nerve injuries are relatively commonplace and primarily these are transection injuries. Transection injury presents a significant challenge clinically for effective repair to regain full functional recovery. Autografts are the current gold standard of peripheral nerve gap repair but have several limitations such as the lack of grafting material, the requirement of a second surgery and mismatched nerve structure (Ray & Mackinnon, 2010). Therefore, biodegradable and biocompatible nerve guidance conduits have been developed as alternatives to replace autograft repair (Kehoe et al., 2012). However, despite the efforts that have been made to improve the structure of the conduit, to embed neurotrophins and growth factors and to deliver cells within the conduit, functional recovery has never reached the same level as seen with autografts for the repair of nerve gaps bigger than 1 cm (Moore et al., 2009). Furthermore, the peripheral nerve regeneration capacity declines in older patients as well as in individuals with complications such as chronic denervation and diabetic neuropathy (Rosenberger et al., 2020; Jessen & Mirsky, 2019; Painter, 2017), which further increases the difficulty of finding an effective treatment.

Migrating Schwann cells are the key cell type in the nerve gap guiding regenerating axons into the distal nerve stump. Therefore, the relationships between Schwann cells and regenerating axons in the nerve bridge are of particular interest to clinicians and researchers in order to develop novel therapeutic strategies for effective peripheral nerve gap repair. Currently, key signals that activate Schwann cell migration or that Schwann cells use to direct axon regeneration have not been fully studied in vivo using the nerve transection model. Understanding these two facets of Schwann cell biology are the most important steps toward the development of new approaches for repairing such nerve gaps. Use of the in vivo sciatic nerve transection research model will be an important approach to identify these key signals in future studies.

DATA AVAILABILITY STATEMENT

This is a review article and all the data has been published in this article. Original images are available upon reasonable request by emailing to the corresponding author.

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